

OBSERVATIONS ON THE FOOD HABITS OF ENTAMOEBA MURIS AND ENTAMOEBA RANARUM

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I. INTRODUCTION

While studying nuclear structure and nuclear division in *Entamoeba muris* (Wenrich, 1940), casual observations indicated that there were food preferences among different individuals and among different populations of these amoebae. Further investigation showed that these amoebae often developed temporarily differentiated ingestion tubes which stain intensely with Heidenhain's haematoxylin. A preliminary report on these observations was made at the Marine Biological Laboratory and an abstract published (Wenrich, 1939). Subsequent studies extended the survey to other sets of slides showing *E. muris* from both rats and mice and similar conditions were then discovered for *E. ranarum* from frogs and toads. The present more extended and illustrated report covers the entire set of observations.

These studies have been made partly at the Marine Biological Laboratory and partly at the University of Pennsylvania. They have been made entirely on fixed and stained slides. A variety of fixing and staining agents have been employed in preparing the slides from caecal and rectal contents but the majority of the smears have been fixed in Schaudinn's sublimate-alcohol-acetic and stained with Heidenhain's haematoxylin.

The rats and mice from which slides were made were secured from a variety of sources but a good many rats were obtained from the Wistar Institute and to The Institute, and especially to Doctor Helen Dean King, grateful acknowledgment is made. Acknowledgment is also made to the staff of the Department of Zoölogy of the University of California at Berkeley, for aid in securing frogs and tadpoles and for facilities for their examination. Most of the observations on *Entamoeba ranarum* were made on material from *Rana pipiens* examined at the University of Pennsylvania.

OBSERVATIONS

Entamoeba muris

Entamoeba muris lives in the caecum of rats and mice. Of the more than 500 rats and more than 100 mice that have been examined during the past twenty-five years, relatively few have had amoebae in sufficient numbers to warrant detailed study.

The more striking results of this study of the food habits of *E. muris* are: (1) that there is a great diversity in the kinds of objects selected as food; (2) that some individuals may limit themselves, for a time at least, to a single type of food with which they may engorge themselves, while others may engulf a considerable variety of food objects; (3) that certain populations of amoebae, that is, those from a single host rat or mouse, may show strong tendencies to select one kind of food material, while in other populations, such tendencies are not manifested; and (4) that differentiated ingestion tubes are developed for the ingestion of some kinds of food.

The food objects fall into two natural divisions or groups; those of a plant nature and those of an animal nature. The former group is represented by a great variety of bacterial organisms, a few of which are illustrated by Figs. 1 to 4 and 28, yeasts (Fig. 5), *Blastocystis* (Fig. 12), plant filaments (Figs. 6, 24-27), all of which are apparently resident within the caecum; and starch grains (Figs. 7, 13, 15-18) from the host's diet. Animals are represented by the other Protozoa resident in the host's caecum and small intestine and by various types of cells derived from the host. Among the Protozoa are the trichomonads (Figs. 11, 14, 29-32), *Chilomastix* and *Hexamitus pulcher* from the caecum; and *Giardia* (Figs. 10, 13) and *Hexamitus muris* which come down from the small intestine. Host cells found ingested were erythrocytes (Fig. 8), leucocytes (Figs. 9, 19), and epithelial cells.

Diversity of food preferences among individuals of a population as well as instances of specialization by individuals are illustrated by Figs. 3, 6, 7, and 10, all from the caecum of one mouse. Figures 15, 17, 18, 20-27 are also from the same population. Preferences by individuals are illustrated on Plate I, where each amoeba has filled its cytoplasm with one kind of food. Figures 1 to 7 show ingested plant materials so that these individuals might be considered to have been "herbivorous," at least temporarily. Figures 8 to 11 illustrate individuals which were "carnivorous" at the time that they were killed, and the amoebae in Figs. 13 and 14 could be called "omnivorous" since they contain food objects of both plant and animal nature.

Population food preferences are occasionally noteworthy. The fusi-

form bacillus shown in Fig. 1 is probably the most common food object seen and many populations show a high percentage of their members containing this organism. The colonial species seen in Fig. 2 is sometimes given preference by a population. On the set of slides from which this figure was drawn, about 65 per cent of the amoebae had ingested one or more of these colonies, most of which, however, were much smaller than the one shown in Fig. 2 (cf. Fig. 14). Two quite different types of diplococcoid species are shown in Figs. 3 and 4. These are not uncommon food inclusions, but it is unusual to see so many of either kind in any single individual. Many other types of bacteria are found in the amoebae, but they have not been identified or drawn.

Yeasts are not uncommon food objects, but specialization on yeasts, as shown in Fig. 5, is uncommon. Several populations were found in which ingested filaments were more than occasionally seen, although the proportion of individuals enclosing filaments in any one population was never more than 2 or 3 per cent. Starch grains were not very commonly seen, although a number of populations included individuals which had ingested such grains.

Populations with ingested host cells were uncommon. Epithelial cells inside amoebae were seen only on a few occasions. Erythrocytes taken as food were noted in only two populations which were from mice. In one the number of individuals showing erythrocytes was greater than in the other, but in both there was a tendency for the same individual to ingest several red cells, as illustrated by Fig. 8. Ingestion of leucocytes was also uncommon and the one population in which a number of

PLATE I

Figs. 1-11 showing examples of specialization by individual amoebae. Figs. 3, 6, 7, and 10 are from the same mouse. Figures 8 and 9 are also from mice. Figures 1, 2, 4, 5, and 11 are from rats.

Figs. 1-7. Examples of "herbivorous" food preferences.

FIG. 1. Amoeba filled with fusiform bacillus—the most common type of food.

FIG. 2. Amoeba containing a large colonial organism.

FIG. 3. Amoeba containing many small diplococcoid bacteria.

FIG. 4. Amoeba with large diplococcoid species.

FIG. 5. Amoeba with a dozen yeast cells.

FIG. 6. Amoeba with long coiled filament.

FIG. 7. Amoeba containing six starch grains. Note deeply-stained granules on side of two of them.

Figs. 8-11. Examples showing "carnivorous" habits.

FIG. 8. Amoeba showing five erythrocytes; one more was under the nucleus.

FIG. 9. Amoeba with four leucocytes.

FIG. 10. Amoeba with three specimens of *Giardia*.

FIG. 11. Amoeba with eight specimens of *Trichomonas muris*.

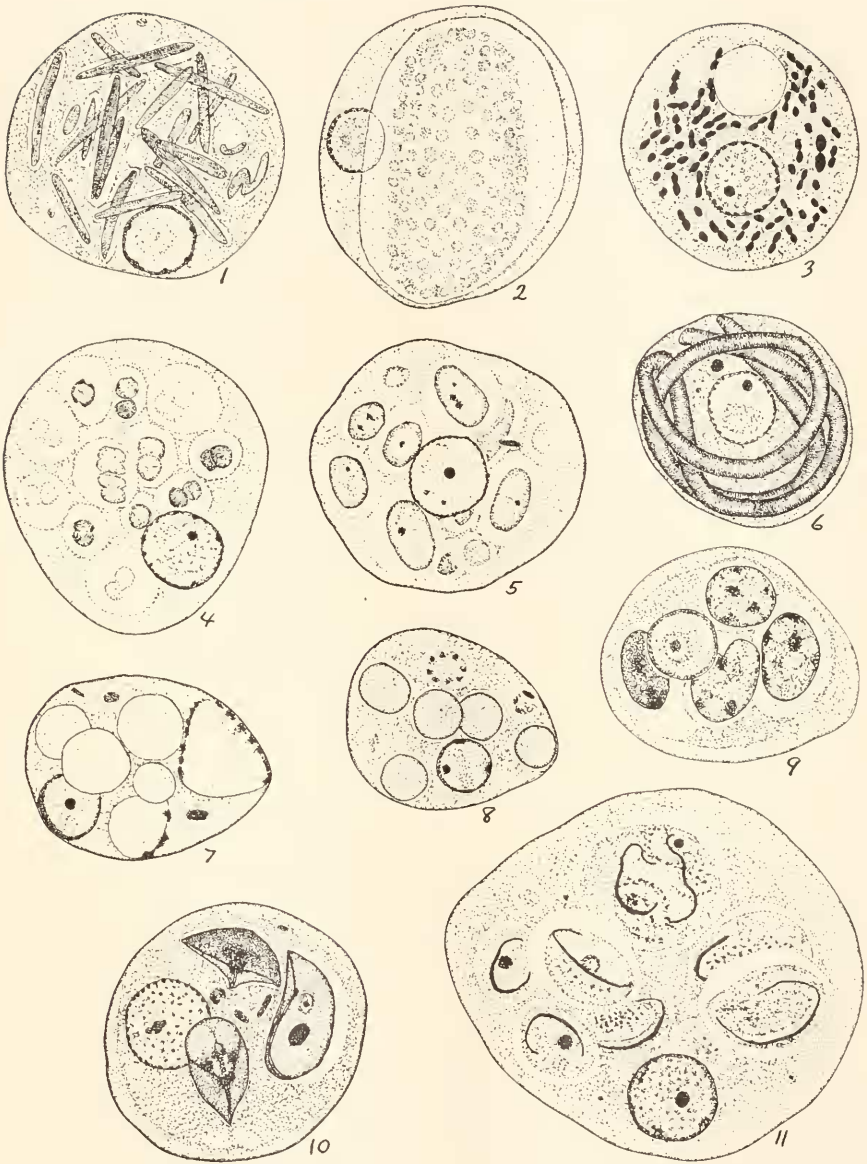


PLATE I

All figures are from fixed and stained slides. They have been drawn with the aid of a camera lucida at an initial magnification of $\times 3000$ and reduced about one-third in printing. Figures 1-33 are of *Entamoeba muris* and Figs. 34-42 are of *E. ranarum*.

amoebae were found with ingested leucocytes was in a mouse. Individual preference is illustrated in Fig. 9.

Among the ingested Protozoa, *Trichomonas muris* was the most common. In many populations it was rarely seen as a food object, while in others it was the population preference. In one count from a slide from a rat about 80 per cent showed one or more trichomonads in various stages of digestion. Individual preferences for this flagellate to the exclusion of other food objects were common in such populations. *Chilomastix bettencourti*, *Hexamitus pulcher* and *Hexamitus muris* were seen within the amoebae on only a few occasions. *Giardia* was seen more frequently but was not commonly observed. In the population from a mouse, from which Fig. 10 was taken, between 3 and 4 per cent showed one to three individuals of this flagellate. In this population only the trophic stages of *Giardia* were ingested, although the cysts were available.

Ingestion Methods.—*Entamoeba muris* apparently adopts somewhat different methods for the intake of food, depending upon the nature of the material to be ingested. In the case of starch, it appears from Figs. 15 to 18 that food cups are formed which are just big enough to take in the granules with no vacuolar space between the food body and the cytoplasm. The absence of a vacuole around starch grains is also indicated in Figs. 7 and 13. In some cases (Figs. 7 and 13), deeply-stained bodies are seen in the cytoplasm which is in contact with the starch grain. These bodies are absent in other cases (Figs. 15–18) and in Fig. 7 only

PLATE II

Figs. 12, 14, from rat. Figs. 13, 15, 17, 18, 20 and 21–33 from one mouse. Figs. 16 and 19 from another mouse.

FIG. 12. Amoeba with specimen of *Blastocystis*.

FIG. 13. Amoeba containing two starch grains, a specimen of *Giardia* and several bacteria.

FIG. 14. Amoeba containing *T. muris*, six colonial organisms and two bacilli.

FIG. 15. Amoeba with starch grain half ingested. Note that edge of food cup and cytoplasmic layer in contact with starch are deeply stained.

FIG. 16. Amoeba containing large starch grain.

FIGS. 17 AND 18. Show ingestion of starch grain almost completed. Note deeply-stained edges of closing-in pseudopodia.

FIG. 19. Amoeba with two partly ingested leucocytes. Note constriction of leucocytes.

FIG. 20. Amoeba with empty food cup. Wall of cup composed of denser cytoplasm but not deeply stained.

FIG. 21. Amoeba with empty food cup. Wall of cup deeply stained.

FIG. 22. Amoeba with food cup turned "wrong-side-out."

FIG. 23. Amoeba with food cup with partly ingested food object and deeply-stained walls.

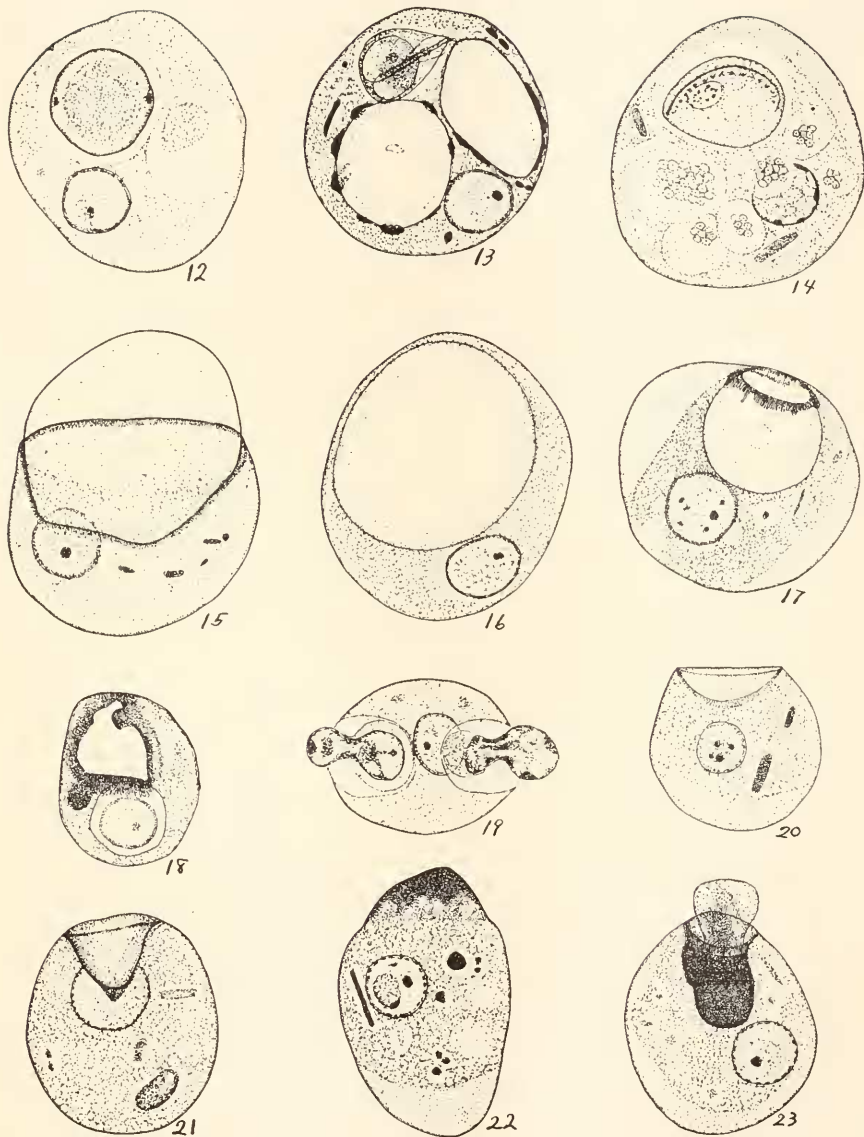


PLATE II

two of the six grains show them. Their nature is problematical, but it is assumed that they are related to digestion. In a number of cases the edges of the advancing pseudopodia which were closing in on a starch grain were deeply stained (Fig. 15) and more especially during the later stages of the enclosing process (Figs. 17 and 18).

In a number of instances, empty food cups were seen (Figs. 20 and 21) and the walls of these cups were obviously composed of denser cytoplasm which might (Fig. 21) or might not (Fig. 20) stain intensely. The condition shown in Fig. 22 is interpreted to be a food cup turned "wrong-side-out." In Fig. 23 a food cup is shown with a partially ingested object and with deeply-stained walls. The middle part of this tube-like cup is more deeply stained than the rest, suggesting greater thickness or greater density.

Figure 19 shows a small specimen of *E. muris* which was fixed while ingesting simultaneously two leucocytes, one at each side. Constriction of the leucocytes is indicated, but the edges of the two food cups are not deeply stained.

Ingestion of filaments was in some cases (Figs. 24 and 27), but not in others (Figs. 25 and 26), accompanied by the formation of deeply-stained ingestion tubes in connection with the ingestion cones. In Fig. 25 a filament has been surrounded at a region away from either end. Ingestion cones were formed and were advancing along the filament in both directions. In Fig. 27, an especially long, deeply-stained food tube is shown. From the left end of this tube and proceeding toward the right, there are three thickenings in the wall of the tube on alternate sides, suggesting a spiral band of more intensely staining material.

A definite "mouth" or ingestion cone and deeply staining "pharynx"

PLATE III

Figs. 24-27 from same mouse. Fig. 30 from another mouse. Figs. 28, 29, 31-33 from rats.

FIG. 24. Amoeba with long filament partly coiled inside. Note deeply-stained ingestion cone and "pharynx."

FIGS. 25 AND 26. Amoeba with partly ingested filaments; ingestion cones not deeply stained.

FIG. 27. Amoeba with partly ingested filament; long, deeply-stained "pharynx."

FIG. 28. Differentiated "mouth" and "pharynx" with partly ingested bacillus.

FIG. 29. Amoeba with ingestion of *T. muris* almost completed. Note deeply-stained "mouth" followed by undifferentiated food cavity with deeply-stained constriction farther in.

FIG. 30. Amoeba ingesting *T. muris* through differentiated "pharynx."

FIGS. 31 AND 32. Amoebae with apparently broken ingestion tubes, due to traumatism. In Fig. 32 the lower tube is all inside and is possibly a constriction tube.

FIG. 33. Amoeba with internal constriction tube.

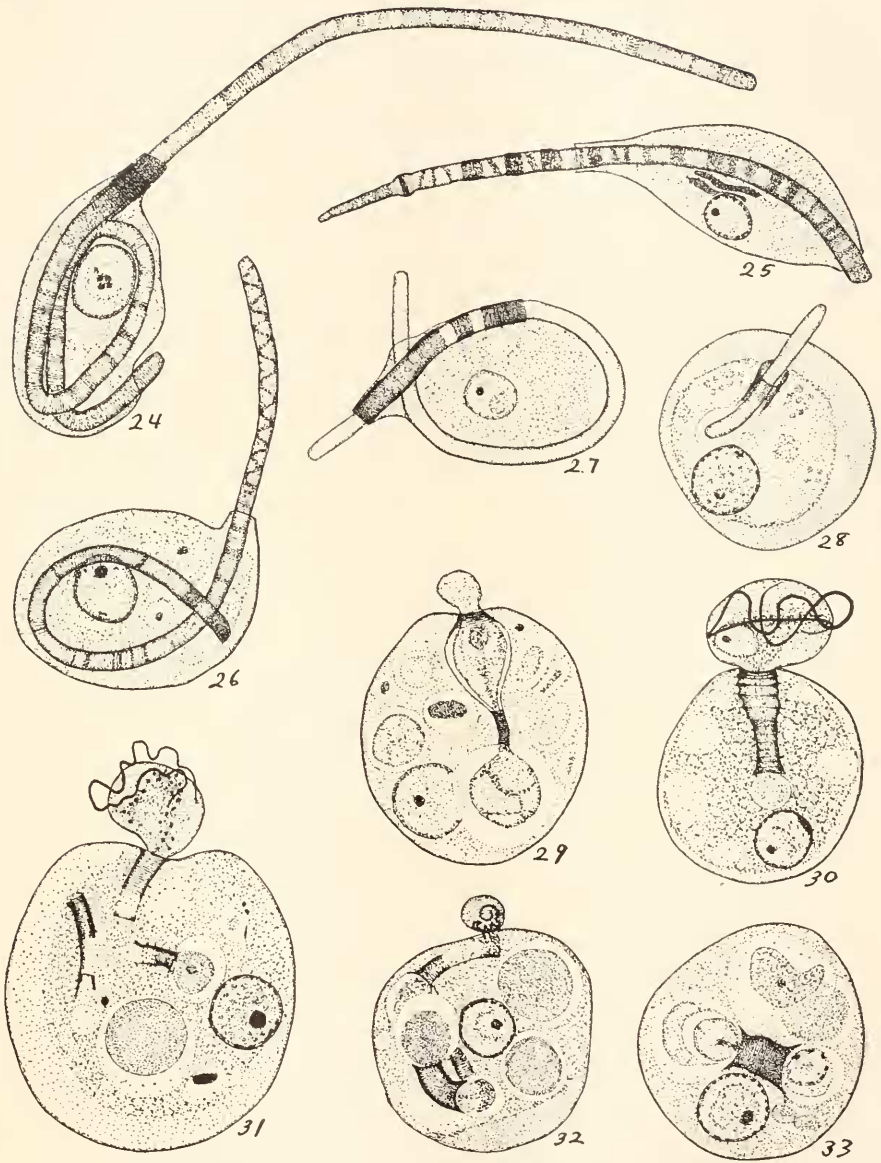


PLATE III

for the ingestion of rod-shaped bacteria are shown in Fig. 28. A number of narrow tubes of this type were seen containing partly ingested bacterial rods.

Trichomonas is apparently ingested by differentiated tubes. A typical case is illustrated in Fig. 30 and many variations of this picture have been seen. In one case the axostyle had been drawn into such a tube while the remainder of the victim remained outside. In another case the anterior flagella had been taken in and the prey had descended a short distance "head first." In still another case the posterior flagellum had been ingested ahead of the rest of the animal. Apparently the amoeba is able to "seize" the flagellate at any point on the latter's surface. In one instance two converging tubes were attached to one trichomonad. The ingestion tube varies in length, up to more than half the width of the amoeba. In some cases the diameter varies in different regions (Figs. 29 and 30). In Figs. 31 and 32 traumatic fragmentation of ingestion tubes is indicated. The amoeba illustrated by Fig. 31 showed definite signs of injury. In a few cases trichomonads were found partly incased in broad food cups, such as shown in Figs. 21 and 23. It is possible that early stages of ingestion may involve such food cups, to be followed by the gradual development of the differentiated tubes such as seen in Fig. 30.

There is evidence that similar tubes are employed to break up food masses, as illustrated in Fig. 33. It is possible that in Fig. 32 a combination of an ingestion tube and a constriction tube is indicated.

Entamoeba ranarum

The finding of the conditions just described for *Entamoeba muris* led to an examination of smears made from the rectum of frogs and

PLATE IV

All figures of Entamoebae from frogs. Figs. 34, 35, 36, 38, 39, and 40 of *E. ranarum* from *Rana pipiens*. Figs. 37, 41, 42, of possibly different species, from California frog, *Rana draytonii*.

FIG. 34. Amoeba with a dozen specimens of *Hexamitus*.

FIG. 35. Amoeba with a specimen of *Trichomonas augusta*.

FIG. 36. Amoeba with four specimens of *Chilomastix*.

FIG. 37. Amoeba with five host cells.

FIG. 38. Amoeba with partly ingested filament; two deeply-stained "pharyngeal" regions.

FIG. 39. Amoeba with partly ingested short filaments; not deeply-stained "pharynx."

FIG. 40. Amoeba containing one host cell nucleus and a partly ingested second host cell nucleus.

FIG. 41. Amoeba with empty food cup. (cf. Figs. 20, 21.)

FIG. 42. Amoeba showing constriction of food inside cytoplasm.

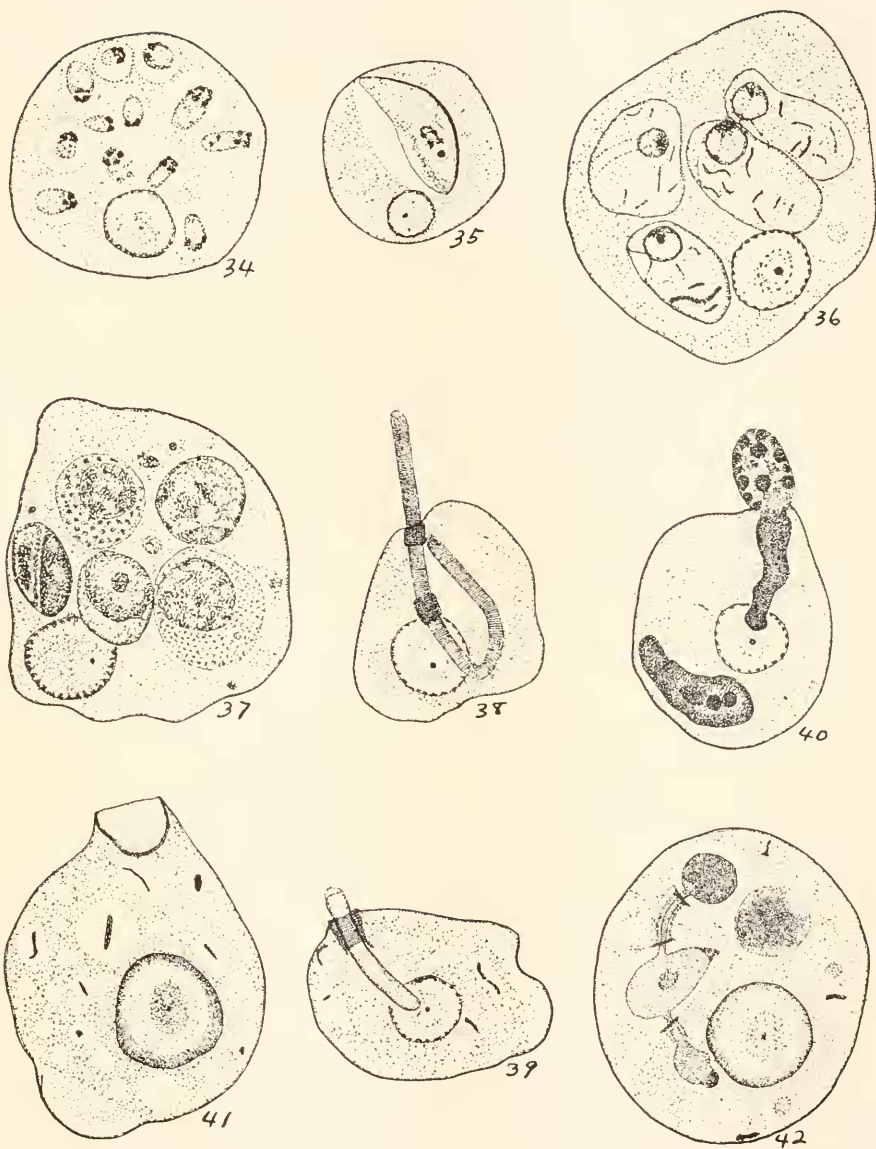


PLATE IV

toads. Here similar conditions were observed for *E. ranarum*. Some of these conditions are illustrated in Figs. 34-42. Individual specialization is indicated by Figs. 34-37. The amoeba in Fig. 34 contains twelve specimens of *Hexamitus*. The population in this case tended to favor *Hexamitus* as a diet, since 17 per cent had ingested one or more individuals. On the same slide 3 per cent of the amoebae contained trichomonads. In a count on a slide showing another population, 50 per cent of the amoebae contained *Hexamitus* as food. The population on this other slide showed diversity of choice, however, as indicated by the ingestion of filaments (Figs. 38 and 39) and host cell nuclei (Fig. 40). In another frog, *Chilomastix* was favored by a considerable number of the amoebae (Fig. 36).

On slides from the California frog, *Rana draytonii*, a large majority of the amoebae contained host cells, apparently leucocytes, although some may have been erythrocytes (Fig. 37). As many as twelve such cells were counted in a single amoeba. A more balanced diet was represented by an amoeba with four host cells and four individuals of the plant, *Blastocystis*. (The amoebae from this species of frog have a nuclear structure considerably different from that typical of *E. ranarum*, and may therefore be a different species.) In a similar amoeba from a California tadpole, a starch grain was seen.

The methods of ingestion employed by these amoebae from frogs and toads are apparently the same as those employed by *E. muris*. In the ingestion of filaments, the formation of ingestion tubes with deeply-stained annular thickenings is shown in Fig. 38. A short differentiated "pharynx" is shown in Fig. 39. Figure 41 shows an empty food cup similar to that seen in *E. muris* (cf. Figs. 20, 21). Constrictions for the breaking up of food masses are shown in Fig. 42, where there are two constrictions being applied simultaneously to a single food body. An internal constriction tube similar to that shown in Fig. 33 for *E. muris* was also seen on the same slide as that from which Fig. 42 was taken. Altogether, the food habits of *E. ranarum* are quite parallel to those of *E. muris*.

DISCUSSION

Most of the extensive literature dealing with the feeding activities of amoebae is concerned with free-living species, there being relatively few reports on the food habits of those that are parasitic. Since the present study has been limited to fixed and stained specimens, the behavior aspects must be inferred, and an extensive discussion of amoeboid nutrition would be inappropriate. However, some interesting interpretations

can be made and their relation to existing literature can be noted. The following items seem worthy of attention here: (1) the diversity of food objects ingested; (2) preferences of individuals and populations for certain kinds of food; (3) methods of ingestion; (4) the breaking up of food bodies after their ingestion; and (5) the appearance of secretion bodies in contact with ingested starch grains.

Diversity of Food Materials Ingested.—Most of those who have studied *Entamoeba muris* have remarked upon the variety of food objects in the cytoplasm of the amoebæ. This diversity has been compared to that frequently mentioned for *E. coli* from man. In amoebæ from the caecum of mice, Wenyon (1907) noted bacteria of various kinds, *Trichomonas*, *Giardia*, *Hexamitus* and its cysts, yeast cells, and epithelial cells. Kessel (1924) noted the inclusion of *Chilomastix* and smaller amoebæ besides different kinds of bacteria, and Wang and Nie (1934) state that ingested food consists mainly of starch grains, intestinal bacteria and plant debris. To these lists the present study adds *Blastocystis*, long plant filaments, and host erythrocytes and leucocytes.

A similar diversity of food inclusions in *E. ranarum* was noted in the present study. Dobell (1909) made few comments on the food of this species but remarked that when blood corpuscles and broken-up epithelial cells were available in the large intestine the amoebæ readily ingested them. In the present study host cells, not clearly identifiable, but possibly including both erythrocytes and leucocytes, were conspicuous food objects in the amoebæ from *Rana draytonii*.

Individual and Population Food Preferences.—The tendency for a single amoeba to ingest repeatedly the same kind of food object is well known for *E. histolytica*, individuals of which may contain as many as thirty to forty erythrocytes at one time; and, in cultures, these amoebæ may engorge themselves with starch grains. Frye and Meloney (1936) noted that in cultures this species varied considerably in its tendency to ingest erythrocytes, depending upon the conditions in the medium with which the amoebæ were surrounded. Pavlova (1938) has confirmed some of these results and states further that the capacity of *E. histolytica* to ingest red cells depends upon the pH of the medium, the capacity being greatest at pH values between 5.6 and 6.5. Semenov (1938) reported that ingestion of erythrocytes did not take place unless the latter adhered to the surface of the amoebæ. It is reasonable to suppose that *E. muris* and *E. ranarum* capture bacteria and active flagellates by an initial adhesion of the latter to the surface of the amoebæ, and that this adhesion would, in turn, be controlled by various external and internal factors. One wonders if such factors would be sufficiently limited or specific in their effects to explain repeated ingestion of one kind of food

body by an individual amoeba. If this were so, then the preference of a large percentage of some populations for one kind of food might be similarly explained. However, the divergence of choice commonly exhibited within a population would indicate that individuals tend to vary among themselves as to their physiological state. It is doubtful if population preferences represent racial, that is, genetic, differences, although such a possibility cannot be ignored.

Methods of Ingestion.—Much has been written about the methods by which amoebae take food into their bodies, but Ivanić (1933) was apparently the first to call particular attention to the formation of a "cytostome" and accompanying tube sufficiently differentiated to stain deeply with iron hematoxylin. He first noted such structures in *Amoeba vespertilio*, *Amoeba iuvenalis* and an unnamed species of *Hartmanella*, but extended the observations to *Amoeba entzi* (1936), *Hartmanella maasi* (1936a) and *H. blattae* (1937). I have also seen a deeply-stained (iron hematoxylin) ingestion apparatus in two different small free-living amoebae of the *Hartmanella* type, where the "cytostome" was funnel-shaped, the funnel opening outwardly. On one slide showing these amoebae nearly every individual displayed from one to a dozen of these funnels at various points on the periphery.

In *E. muris*, ingestion methods seem to be much more diversified, apparently adapted to different kinds of food, but they include the formation of specialized tubes which stain intensely with iron hematoxylin.

In many of his illustrations Ivanić shows, proximal to the "cytostome," capacious vacuoles and speaks of food bodies as being drawn into them. Some of the conditions seen in the present study would lend support to this interpretation. In Fig. 19 the concentration of the more fluid cytoplasm at the inner ends of the partly ingested leucocytes together with the constriction at the "mouth" suggests suction. Suction is also suggested in the ingestion of *Trichomonas* by *E. muris*; here various portions, anterior flagella, posterior flagellum, etc., can be identified as having gone down the "pharynx" in advance of other portions, and often a rounded globule of trichomonas protoplasm occupies an internal vacuole while other portions of the flagellate remain outside and the two parts remain connected through the tube-like "pharynx" (Figs. 29, 30). The enlarged vacuoles into which the short filaments are entering in Figs. 28 and 39 suggest the same thing. Semenov (1937, 1938) found that *E. histolytica* frequently extracted the nuclei from frog and chick erythrocytes although sometimes ingested fragments might include some cytoplasm. It is difficult to understand how suction

can be developed within an amoeboid cell, but the evidence at hand favors that interpretation.

The ingestion of starch appears to take place by simple extension of pseudopodia over the food object and in contact with it (Figs. 15, 17, 18). This method resembles that frequently reported for free-living amoebae during the act of ingesting starch or other solid bodies. Brug (1928) saw a living specimen of *E. histolytica* enter a group of starch grains and emerge two or three minutes later with four larger and two smaller grains in its cytoplasm, but he did not see the method of intake.

E. muris and *E. ranarum* apparently ingest filaments in a manner similar to that described for free-living species in such classical papers as those of Leidy (1879) and Rhumbler (1898); and more recently Comandon and Fonbrune (1936), have recorded their observations with motion pictures. Ivanić (1933) showed that ingestion of filaments by *A. vespertilio* is accompanied by deeply stainable thickenings along the ingestion tube and the present study reveals similar conditions for *E. muris* and *E. ranarum* (Figs. 24, 27, 38). That a differentiated tube, such as shown in Fig. 27, is fairly stable—for a time at least—is indicated by the finding of a similar tube attached to a bent filament but with the remainder of the amoeba missing—probably having been torn off during the smearing process. It is probable that Fig. 25 represents an early stage in the bending of the filament, a process which might well result in the condition seen in Fig. 27. It is interesting that Figs. 25 and 26 do not show the deeply-stained walls of the ingestion tube that are seen in Figs. 24 and 27. It is doubtful if these differences are the result of variations in the destaining process, since Figs. 24 and 26 were drawn from the same slide. Stainability seems to vary with density of protoplasm and the density is doubtless correlated with degree of contraction.

Peristaltic contractions may be indicated by the successive thickenings on alternate sides of the "pharynx" shown in Fig. 27. Comandon and Fonbrune (1936), employing motion pictures, record the observation of waves of contraction along the ingestion cone surrounding a filament in *A. verrucosa*. Peristaltic action during ingestion of trichomonads by *E. muris* may also be indicated by the differences in diameter of the "pharynx" shown in Figs. 29 and 30.

The Breaking Up of Food Bodies After Their Ingestion.—The ability of amoebae to break up food masses into smaller units has been noted by a number of observers, for example by Leidy (1879) and Penard (1912). More recently Entz (1925) has provided a good description of successive constrictions of food objects as seen in *Amoeba vespertilio*; and, in a later paper (1932) he reviewed the literature show-

ing instances of the breaking up of food masses in both amoebae and ciliates, and also in the flagellate, *Collodictyon*. Ivanić (1936) described the constriction of ingested food masses, sometimes several such constrictions taking place simultaneously; and Mast (1938) reported the breaking up of ingested *Colpidium* in the cytoplasm of *Amoeba proteus*.

The present record seems to be the first for the breaking up of food in a species of *Entamoeba* and none of the observers referred to above have reported the presence of deeply-stained constriction tubes, such as shown in Figs. 33 and 42. It is possible that the deeply-stained tube in Fig. 33 represents a "pharynx" which has persisted after the prey was ingested, although the vacuole at each end does not suggest that interpretation; and such an interpretation would not be applicable to the condition seen in Fig. 42.

Wenyon (1907) speaks of seeing several specimens of *Trichomonas* in a single vacuole in *E. muris* (see his Fig. 1). On the slides used in the present study, flagellates, or their fragments, were almost always in segregated vacuoles. However, large vacuoles, each containing many bacteria, were sometimes seen and one wonders if fusion of vacuoles may take place as well as their subdivision. Ivanić, however, believed that a succession of objects would be taken in through a single "cytostome."

Digestive Granules in Contact with Ingested Starch Grains.—Figure 13 shows a specimen of *Entamoeba muris* containing two starch grains, each of which has deeply-stained masses at its periphery. Figure 7 shows an amoeba with six starch grains and similar stained bodies are seen at the sides of two of them. That it takes some time for such bodies to appear is indicated by their absence in Figs. 15 to 18 where starch grains are being ingested, and also their absence from four of the six grains in Fig. 7. It seems reasonable to assume that these bodies in contact with food represent secreted material having a digestive function. Very similar bodies are shown by MacLennan (1936) for food bodies in *Ichthyophthirius* and he identifies them as elements of the vacuome since they react positively to neutral red and to the Kolatchev-Nassanov method for impregnation of Golgi material. Volkonsky (1934) shows similar neutral red staining bodies outside starch grains ingested by a large granulocyte of *Phascolosoma*, and also by a choanocyte of *Clathrina coriacea*. In his general review of cytoplasmic inclusions in Protozoa, MacLennan (1941) refers to such bodies as digestive granules.

Various observers have denied to free-living amoebae the capacity to digest starch. However, the avidity with which *E. histolytica* and other endamoebae ingest this form of carbohydrate is well established. It

should not be surprising therefore, if, as in the other cells referred to, digestive secretions should be elaborated by such amoebae for the digestion of starch.

SUMMARY

On the basis of observations on fixed and stained slides showing *Entamoeba muris* and *E. ranarum*, the following observations and interpretations have been made.

In general, these species of *Entamoeba* show great diversity in the kinds of food ingested. *E. muris* more commonly feeds on a fusiform bacillus but its diet includes many other types of bacteria, *Blastocystis*, yeasts, plant filaments, starch grains, *Trichomonas*, *Chilomastix*, *Hexamitus*, and host erythrocytes, leucocytes and epithelial cells. *E. ranarum* shows a similar range of food objects.

Individuals often select for a time, at least,—a single kind of food, with which they may engorge themselves. Others are more omnivorous in their selection.

Populations from a single host may show decided preferences for one type of food; for example, about 80 per cent of one population of *E. muris* contained one or more specimens of *Trichomonas*.

A diversity of methods of ingestion is indicated. Starch grains are surrounded by enveloping pseudopodia without the formation of a fluid-containing vacuole around them. Trichomonads appear to be drawn through an ingestion tube with walls sufficiently differentiated to stain heavily with iron hematoxylin. Plant filaments are taken in through similar tubes some of which show the deeply-stained walls. There is evidence that differentiated tubes are employed to constrict food bodies into smaller units.

Bodies which stain with iron hematoxylin have been seen in contact with ingested starch grains in *E. muris*. These are interpreted as digestive granules in the sense that this term is used in the review by MacLennan (1941).

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